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(54) Title: HIGH LEVEL EXPRESSION OF PROTEINS

(57) Abstract

The invention features a synthetic gene encoding a protein normally expressed in a mammalian cell or eukaryotic cell wherein at least one non-preferred or less preferred codon in the natural gene encoding the mammalian protein has been replaced by a preferred codon encoding the same amino acid.

```
  1  GAATTCACGC GTAAGCTTGC CGCCACCATG GTGAGCAAGG GCGAGGAGCT
 51  GTTCACCGGG GTGGTGCCCA TCCTGGTCGA GCTGGACGGC GACGTGAACG
101  GCCACAAGTT CAGCGTGTCC GGCGAGGGCG AGGGCGATGC CACCTACGGC
151  AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC CCGTGCCCTG
201  GCCCACCCTC GTGACCACCT TCAGCTACGG CGTGCAGTGC TTCAGCCGCT
251  ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCGAA
301  GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
351  GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
401  AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGGCACAAG
451  CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
501  GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
551  GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC
601  GGCCCCGTGC TGCTGCCCGA CAACCACTAC CTGAGCACCC AGTCCGCCCT
651  GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCCTG CTGGAGTTCG
701  TGACCGCCGC CGGGATCACT CACGGCATGG ACGAGCTGTA CAAGTAAAGC
751  GGCCGCGGAT CC    (SEQ ID NO: 40)
```

What is claimed is:

1. A synthetic gene encoding a protein normally expressed in a eukaryotic cell wherein at least one non-preferred or less preferred codon in the natural gene encoding said protein has been replaced by a preferred codon encoding the same amino acid.

2. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 110% of that expressed by said natural gene in an in vitro mammalian cell culture system under identical conditions.

3. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 150% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

4. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 200% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

5. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least 500% of that expressed by said natural gene in an in vitro cell culture system under identical conditions.

6. The synthetic gene of claim 1 wherein said synthetic gene is capable of expressing said eukaryotic protein at a level which is at least ten times that

expressed by said natural gene in an in vitro cell culture system under identical conditions.

7. The synthetic gene of claim 1 wherein at least 10% of the codons in said natural gene are non-preferred codons.

8. The synthetic gene of claim 8 wherein at least 50% of the codons in said natural gene are non-preferred codons.

9. The synthetic gene of claim 1 wherein at least 50% of the non-preferred codons and less preferred codons present in said natural gene have been replaced by preferred codons.

10. The synthetic gene of claim 1 wherein at least 90% of the non-preferred codons and less preferred codons present in said natural gene have been replaced by preferred codons.

11. The synthetic gene of claim 1 wherein said protein is green fluorescent protein.

12. A method for preparing a synthetic gene encoding a protein normally expressed by eukaryotic cells, comprising identifying non-preferred and less-preferred codons in the natural gene encoding said protein and replacing one or more of said non-preferred and less-preferred codons with a preferred codon encoding the same amino acid as the replaced codon.

Syngp120mn

```
   1  CTCGAGATCC ATTGTGCTCT AAAGGAGATA CCCGGCCAGA CACCCTCACC
  51  TGCGGTGCCC AGCTGCCCAG GCTGAGGCAA GAGAAGGCCA GAAACCATGC
 101  CCATGGGGTC TCTGCAACCG CTGGCCACCT TGTACCTGCT GGGGATGCTG
 151  GTCGCTTCCG TGCTAGCCAC CGAGAAGCTG TGGGTGACCG TGTACTACGG
 201  CGTGCCCGTG TGGAAGGAGG CCACCACCAC CCTGTTCTGC GCCAGCGACG
 251  CCAAGGCGTA CGACACCGAG GTGCACAACG TGTGGGCCAC CCAGGCGTGC
 301  GTGCCCACCG ACCCCAACCC CCAGGAGGTG GAGCTGGTGA ACGTGACCGA
 351  GAACTTCAAC ATGTGGAAGA ACAACATGGT GGAGCAGATG CATGAGGACA
 401  TCATCAGCCT GTGGGACCAG AGCCTGAAGC CCTGCGTGAA GCTGACCCCC
 451  CTGTGCGTGA CCCTGAACTG CACCGACCTG AGGAACACCA CCAACACCAA
 501  CAACAGCACC GCCAACAACA ACAGCAACAG CGAGGGCACC ATCAAGGGCG
 551  GCGAGATGAA CAACTGCAGC TTCAACATCA CCACCAGCAT CCGCGACAAG
 601  ATGCAGAAGG AGTACGCCCT GCTGTACAAG CTGGATATCG TGAGCATCGA
 651  CAACGACAGC ACCAGCTACC GCCTGATCTC CTGCAACACC AGCGTGATCA
 701  CCCAGGCCTG GCCCAAGATC AGCTTCGAGC CCATCCCCAT CCACTACTGC
 751  GCCCCCGCCG GCTTCGCCAT CCTGAAGTGC AACGACAAGA AGTTCAGCGG
 801  CAAGGGCAGC TGCAAGAACG TGAGCACCGT GCAGTGCACC CACGGCATCC
 851  GGCCGGTGGT GAGCACCCAG CTCCTGCTGA ACGGCAGCCT GGCCGAGGAG
 901  GAGGTGGTGA TCCGCAGCGA GAACTTCACC GACAACGCCA AGACCATCAT
 951  CGTGCACCTG AATGAGAGCG TGCAGATCAA CTGCACGCGT CCCAACTACA
1001  ACAAGCGCAA GCGCATCCAC ATCGGCCCCG GGCGCGCCTT CTACACCACC
1051  AAGAACATCA TCGGCACCAT CCGCCAGGCC CACTGCAACA TCTCTAGAGC
1101  CAAGTGGAAC GACACCCTGC GCCAGATCGT GAGCAAGCTG AAGGAGCAGT
1151  TCAAGAACAA GACCATCGTG TTCAACCAGA GCAGCGGCGG CGACCCCGAG
1201  ATCGTGATGC ACAGCTTCAA CTGCGGCGGC GAATTCTTCT ACTGCAACAC
1251  CAGCCCCCTG TTCAACAGCA CCTGGAACGG CAACAACACC TGGAACAACA
1301  CCACCGGCAG CAACAACAAT ATTACCCTCC AGTGCAAGAT CAAGCAGATC
1351  ATCAACATGT GGCAGGAGGT GGGCAAGGCC ATGTACGCCC CCCCCATCGA
1401  GGGCCAGATC CGGTGCAGCA GCAACATCAC CGGTCTGCTG CTGACCCGCG
1451  ACGGCGGCAA GGACACCGAC ACCAACGACA CCGAAATCTT CCGCCCCGGC
```

FIG 1
(SHEET 1 OF 4)

1501 GGGGGCGACA TGCGCGACAA CTGGAGATCT GAGCTGTACA AGTACAAGGT

1551 GGTGACGATC GAGCCCCTGG GCGTGGCCCC CACCAAGGCC AAGCGCCGCG

1601 TGGTGCAGCG CGAGAAGCGC TAAAGCGGCC GC (SEQ ID NO:34)

FIG 1

(SHEET 2 OF 4)

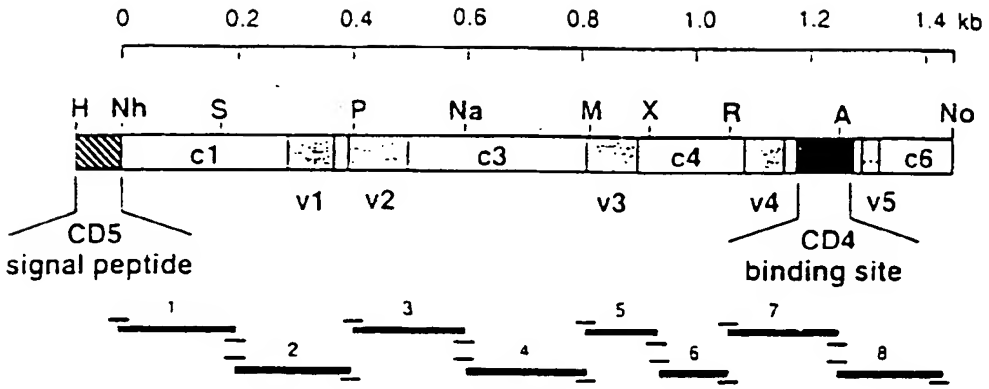
SyngP160mn

```
   1  ACCGAGAAGC TGTGGGTGAC CGTGTACTAC GGCGTGCCCG TGTGGAAGGA
  51  GGCCACCACC ACCCTGTTCT GCGCCAGCGA CGCCAAGGCG TACGACACCG
 101  AGGTGCACAA CGTGTGGGCC ACCCACGCCT GCGTGCCCAC CGACCCCAAC
 151  CCCCAGGAGG TGAACCTGGT GAACGTGACC GAGAACTTCA ACATGTGGAA
 201  GAACAACATG CTGGAGCAGA TGCATGAGGA CATCATCAGC CTGTGGGACC
 251  AGAGCCTGAA GCCCTGCGTG AAGCTGACCC CCCTGTGCGT GACCCTCAAC
 301  TGCACCGACC TGAGGAACAC CACCAACACC AACAACAGCA CCGCCAACAA
 351  CAACAGCAAC AGCGAGGGCA CCATCAAGGG CGGCGAGATG AAGAACTGCA
 401  GCTTCAACAT CACCACCAGC ATCCGCGACA AGATCCAGAA GGAGTACGCC
 451  CTGCTGTACA AGCTGGATAT CGTGAGCATC GACAACGACA GCACCAGCTA
 501  CCGCCTGATC TCCTGCAACA CCAGCGTGAT CACCCAGGCC TGCCCCAAGA
 551  TCAGCTTCGA GCCCATCCCC ATCCACTACT GCGCCCCCGC CGGCTTCGCC
 601  ATCCTGAAGT GCAACGACAA GAAGTTCAGC GGCAAGGGCA GCTGCAAGAA
 651  CGTGAGCACC GTGCAGTGCA CCCACGGCAT CCGCCCCGTG GTGAGCACCC
 701  AGCTGCTGCT GAACGGCAGC CTGGCCGAGG AGGAGGTGGT GATCCGCAGC
 751  GAGAACTTCA CCGACAACGC CAAGACCATC ATCGTGCAGC TGAATGAGAG
 801  CGTGGCAGATC AACTGCACGC GTCCCAACTA CAACAAGCGC AAGCGCATCC
 851  ACATCGGCCC CGGCCGCGCC TTCTACACCA CCAAGAACAT CATCGGCACC
 901  ATCCGCCAGG CCCACTGCAA CATCTCTAGA GCCAAGTGGA ACGACACCCT
 951  GCGCCAGATC GTGAGCAAGC TGAAGGAGCA GTTCAAGAAC AAGACCATCG
1001  TGTTCAACCA GAGCAGCGGC GGCGACCCCG AGATCGTGAT GCACAGCTTC
1051  AACTGCGGCG GCGAATTCTT CTACTGCAAC ACCAGCCCCC TGTTCAACAG
1101  CACCTGGAAC GGCAACAACA CCTGGAACAA CACCACCGGC AGCAACAACA
1151  ATATTACCCT CCAGTGCAAG ATCAAGCAGA TCATCAACAT GTGGCAGGAG
1201  GTGGGCAAGG CCATGTACGC CCCCCCCATC GAGGGCCAGA TCCGCTGCAG
1251  CAGCAACATC ACCGGTCTGC TGCTGACCCG CGACGGCGGC AAGGACACCG
1301  ACACCAACGA CACCGAAATC TTCCGCCCCG GCGGCGGCGA CATGCGCGAC
1351  AACTGGAGAT CTGAGCTGTA CAAGTACAAG GTGGTGACGA TCGAGCCCCT
1401  CGGCGTGGCC CCCACCAAGG CCAAGCGCCG CGTGGTGCAG CGCGAGAAGC
```

FIG. 1
(SHEET 3 OF 4

```
1451  GGGCCGCCAT CGGCGCCCTG TTCCTGGGCT TCCTGGGGCC GCCGGGCAGC
1501  ACCATGGGGG CCGCCAGCGT GACCCTGACC GTGCAGGCCC GCCTGCTCCT
1551  GAGCGGCATC GTGCAGCAGC AGAACAACCT CCTCCGCGCC ATCGAGGCCC
1601  AGCAGCATAT GCTCCAGCTC ACCGTGTGGG GCATCAAGCA GCTCCAGGCC
1651  CGCGTGCTGG CGTGGAGCG CTACCTGAAG GACCAGCAGC TCCTGGGCTT
1701  CTGGGGCTGC TCCGGCAAGC TGATCTGCAC CACCACGGTA CCCTGGAACG
1751  CCTCCTGGAG CAACAAGAGC CTGGACGACA TCTGGAACAA CATGACCTGG
1801  ATGCAGTGGG ACCGCGAGAT CGATAACTAC ACCAGCCTGA TCTACAGCCT
1851  GCTGGAGAAG AGCCAGACCC AGCAGGAGAA GAACGAGCAG GAGCTGCTGG
1901  ACCTGGACAA CTGGGCGAGC CTGTGGAACT GGTTCGACAT CACCAACTGG
1951  CTGTGGTACA TCAAAATCTT CATCATGATT GTGGGCGGCC TGGTGGGCCT
2001  CCGCATCGTG TTCGCCGTGC TGAGCATCGT GAACCGCGTG CGCCAGGGCT
2051  ACAGCCCCCT GAGCTTCCAG ACCCGGCCCC CGGTGCCGCG CGGGCCCGAC
2101  CGCCCCGAGG GCATCGAGGA GGAGGGCGGC GAGCGCGACC GCGACACCAG
2151  CGGCAGGCTC GTGCACGGCT TCCTGGCGAT CATCTGGGTC GACCTCCGCA
2201  GCCTGTTCCT GTTCAGCTAC CACCACCGCG ACCTGCTGCT GATCGCCGCC
2251  CGCATCGTGG AACTCCTAGG CCGCCGCGGC TGGGAGGTGC TGAAGTACTG
2301  GTGGAACCTC CTCCAGTATT GGAGCCAGGA GCTGAAGTCC AGCGCCGTGA
2351  GCCTGCTGAA CGCCACCGCC ATCGCCGTGG CCGAGGGCAC CGACCGCGTG
2401  ATCGAGGTGC TCCAGAGGGC CGGGAGGGCG ATCCTGCACA TCCCCACCCG
2451  CATCCGCCAG ATGGCTCGAGA GGGCGCTGCT G   (SEQ ID NO:35)
```

FIG. 1
(SHEET 4 OF 4)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c4
c6
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binding site
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2
3
4
5
6
7
8

FIGURE 2

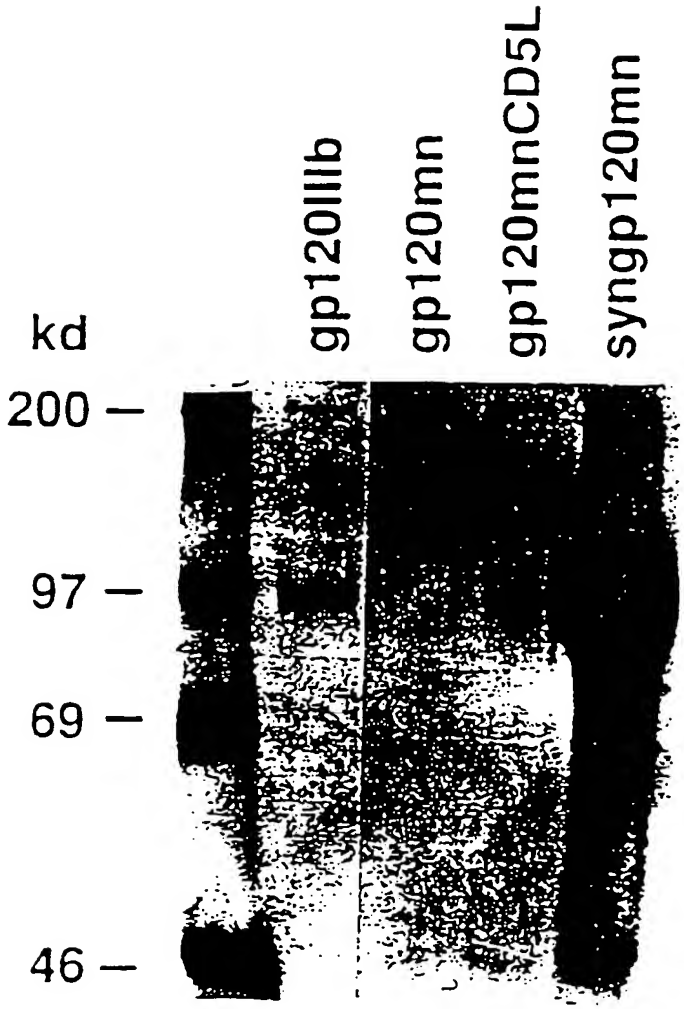
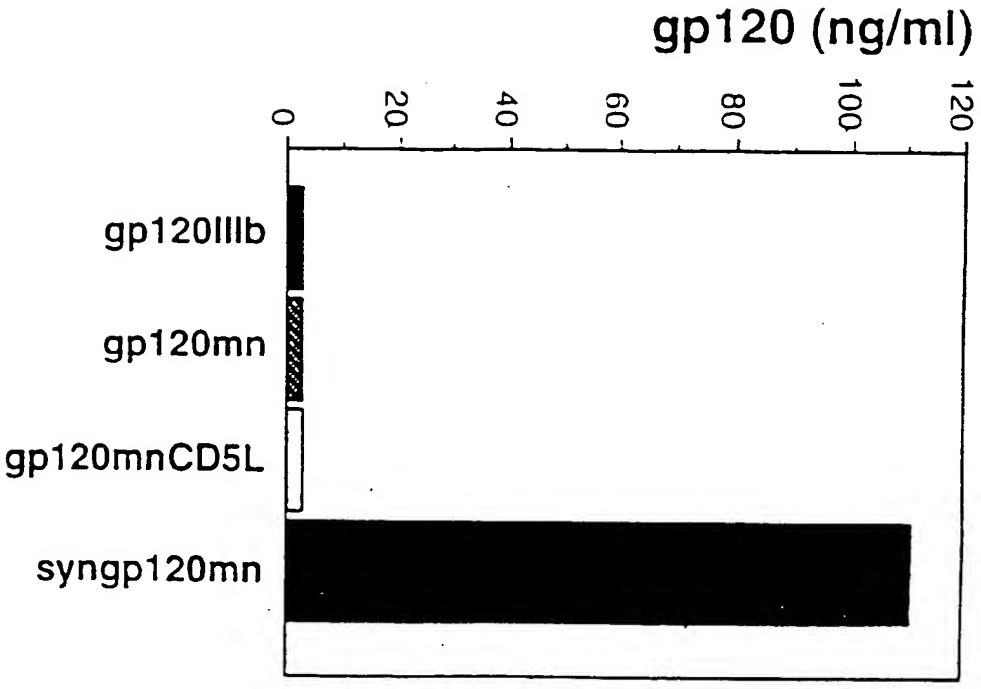


FIGURE 3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120
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gp120mn
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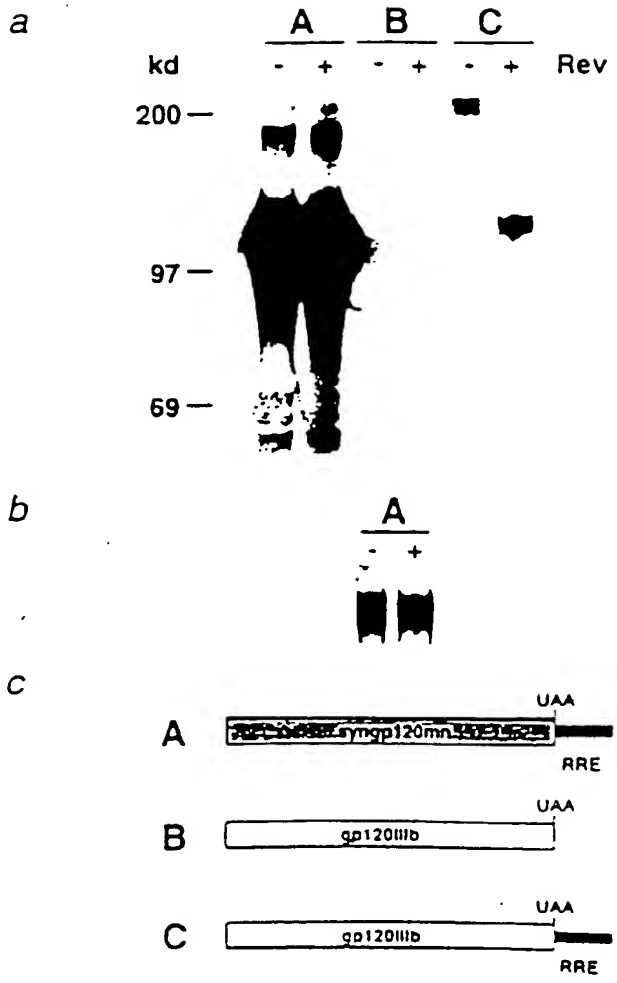
FIGURE 4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B
C
kd
- + - + - + Rev
200
97
69
b
A
- +
c
A
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RRE
B
UAA
gp120IIIb
C
UAA
gp120IIIb
RRE

FIGURE 5

```
                  M   N   P   V   I   S   I   T   L   L   L   S   V   L   Q   M   S   R   G   Q
(SEQ ID NO:36) env→atg aat cca gta ata agt ata aca tta tta tta agt gta tta caa atg agt aga gga caa
(SEQ ID NO:37) wt→ atg aac cca gtc atc agc atc act ctc ctg ctt tca gtc ttg cag atg tcc cga gga cag

                  R   V   I   S   L   T   A   C   L   V   N   Q   N   L   R   L   D   C   R   H
env               aga gta ata agt tta aca gca tgt tta gta aat caa aat ttg aga tta gat tgt aga cat
wt                agg gtg atc agc ctg aca gcc tgc ctg gtg aa  cag aac ctt cga ctg gac tgc cgt cat

                  E   N   N   T   N   L   P   I   Q   H   E   F   S   L   T   R   E   K   K   K
env               gaa aat aat aca cct ttg cca ata caa cat gaa ttt tca tta acg cgt gaa aaa aaa aaa
wt                gag aat aac acc aac ttg ccc atc cag cat gag ttc agc ctg acc cga gag aag aag aag

                  H   V   L   S   G   T   L   G   V   P   E   H   T   Y   R   S   R   V   N   L
env               cat gta tta agt gga aca tta gga gta cca gaa cat aca tat aga agt aga gta aat ttg
wt                cac gtg ctg tca ggc acc ctg ggg gtt ccc gag cac act tac cgc tcc cgc gtc aac ctt

                  F   S   D   R   F   I   K   V   L   T   L   A   N   F   T   T   K   D   E   G
env               ttt agt gat aga ttc ata aaa gta tta aca tta gca aat ttt aca aca aaa gat gaa gga
wt                ttc agt gac cgc ttt atc aag gtc ctt act cta gcc aac ttc acc acc aag gat gag ggc

                  D   Y   M   C   E   L   R   V   S   G   Q   N   P   T   S   S   N   K   T   I
env               gat tat atg tgt gag ctc aga gta agt gga caa aat cca aca agt agt aat aaa aca ata
wt                gac tac atg tgt gaa ctt cga gtc tcg ggc cag aat ccc aca agc tcc aat aaa act atc

                  N   V   I   R   D   K   L   V   K   C   G   G   I   S   L   L   V   Q   N   T
env               aat gta ata aga gat aaa tta gta aaa tgt gga gga ata agt tta tta gta caa aat aca
wt                aat gtg atc aga gac aag ctg gtc aag tgt ggt ggc ata agc ctg ctg gtt caa aac act

                  S   W   L   L   L   L   L   L   S   L   S   F   L   Q   A   T   D   F   I   S
env               agt tgg tta tta tta tta tta tta agt tta agt ttt tta caa gca aca gat ttt ata agt
wt                tcc tgg ctg ctg ctg ctc ctg ctt tcc ctc tcc ttc ctc caa gcc acg gac ttc att tct

                  L   •
env               tta tga
wt                ctg tga
```

FIGURE 6

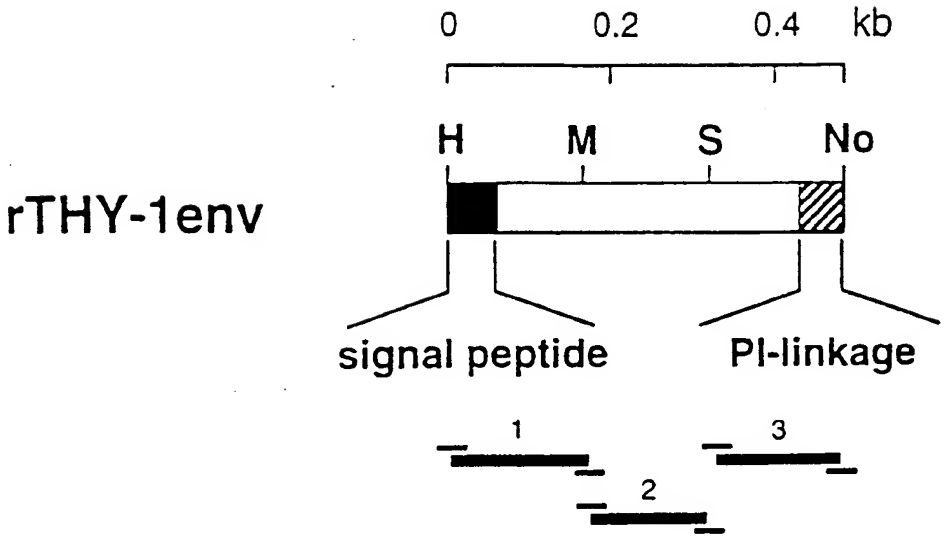
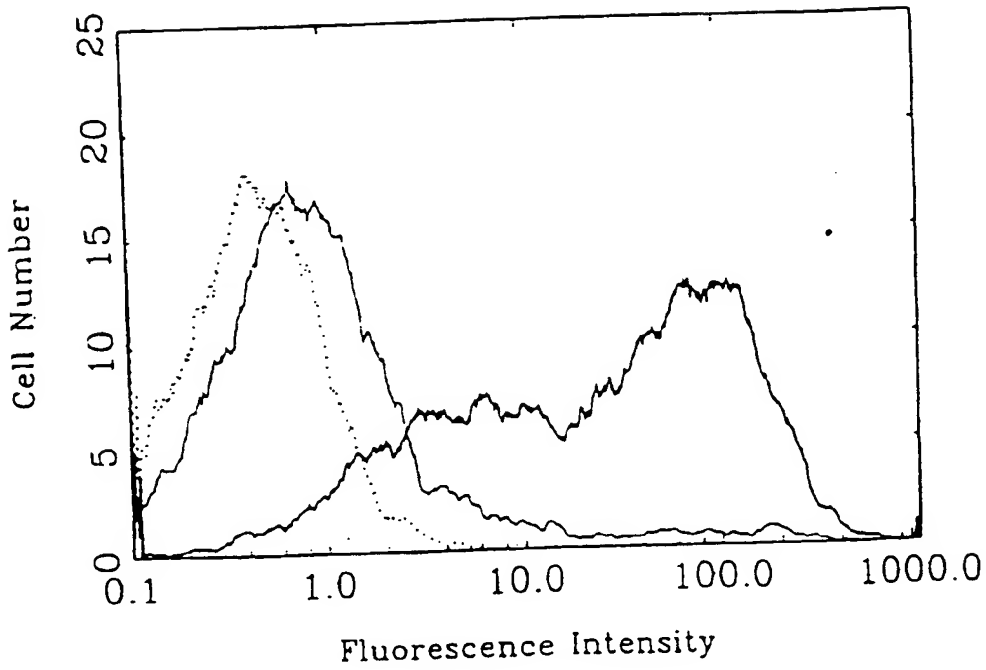


FIGURE 7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10
15
20
25
0.1
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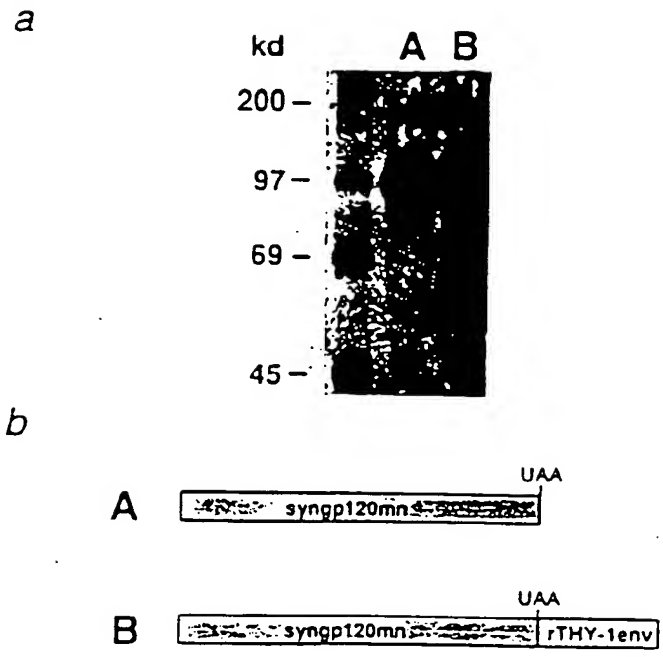
100.0
1000.0
Fluorescence Intensity

FIGURE 8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kd
A B
200
97
69
45
b
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syngp120mn
B
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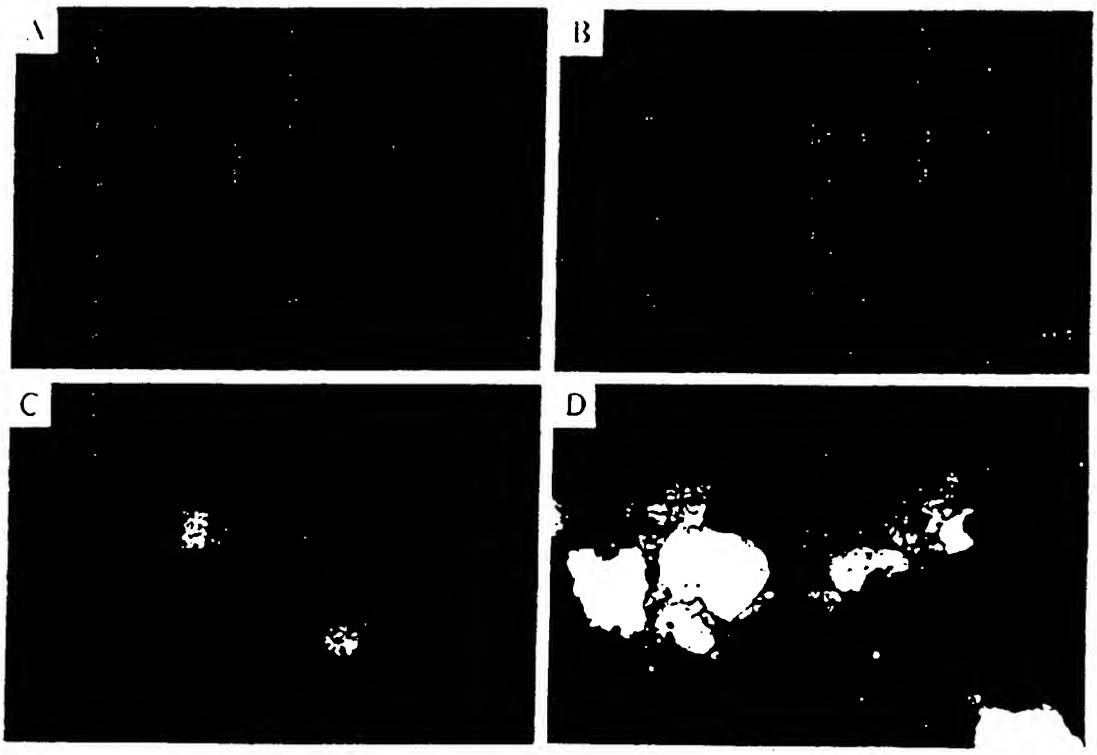
syngp120mn
rTHY-1env

FIGURE 9

FIG. 10



```
   1  GAATTCACGC GTAAGCTTGC CGCCACCATG GTGAGCAAGG GCGAGGAGCT
  51  GTTCACCGGG GTGGTGCCCA TCCTGGTCGA GCTGGACGGC GACGTGAACG
 101  GCCACAAGTT CAGCGTGTCC GGCGAGGGCG AGGGCGATGC CACCTACGGC
 151  AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC CCGTGCCCTG
 201  GCCCACCCTC GTGACCACCT TCAGCTACGG CGTGCAGTGC TTCAGCCGCT
 251  ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCGAA
 301  GGCTACGTCC AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA
 351  GACCCGCGCC GAGGTGAAGT TCGAGGGCGA CACCCTGGTG AACCGCATCG
 401  AGCTGAAGGG CATCGACTTC AAGGAGGACG GCAACATCCT GGGGCACAAG
 451  CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG CCGACAAGCA
 501  GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
 551  GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC
 601  GGCCCCGTGC TGCTGCCCGA CAACCACTAC CTGAGCACCC AGTCCGCCCT
 651  GAGCAAAGAC CCCAACGAGA AGCGCGATCA CATGGTCCTG CTGGAGTTCG
 701  TGACCGCCGC CGGGATCACT CACGGCATGG ACGAGCTGTA CAAGTAAAGC
 751  GGCCGCGGAT CC    (SEQ ID NO: 40)
```

FIG. 11

INTERNATIONAL SEARCH REPORT

Inter. ional application No.
PCT/US96/15088

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/00, 21/04
US CL :536/23.1, 23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, Medline, Biosis, Embase, Scisearch, WPIDS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOLLER et al. HIV1 Integrase Expressed in Escherichia coli From a Synthetic Gene. Gene. 1993, Vol.136, pages 323-328, especially pages 323-327.	1-10, 12
X	SCORER et al. The Intracellular Production and Secretion of HIV-1 Envelope Protein in the Methylotrophic Yeast Pichia pastoris. Gene. 1993, Vol.136, pages 111-119, especially pages 111-118.	1-10, 12
X	HERNAN et al. Human Hemoglobin Expression in Escherichia coli: Importance of Optimal Codon Usage. Biochemistry. 1992, Vol.31, pages 8619-8628, especially pages 8619-8627.	1-10, 12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 NOVEMBER 1996	Date of mailing of the international search report 23 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ENRIQUE D. LONGTON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15088

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WILLIAMS et al. Design, Synthesis and Expression of a Human Interleukin-2 Gene Incorporating the Codon Usage Bias Found in Highly Expressed Escherichia coli Genes. Nucleic Acids Research. 1988, Vol.16, No.22, pages 10453-10467, especially pages 10453-10466.	1-10, 12 ---- 11
X	RANGWALA et al. High-Level Production of Active HIV-1 Protease In Escherichia coli. Gene. 1992, Vol.122, pages 263-269, especially pages 263-268.	1-10, 12
P, X	US 5,464,774 A (BAIRD et al.) 07 November 1995 (07/11/95), see entire document, especially insert at top of columns 13 and 14; column 7, lines 27-51.	1-10, 12
Y	INOUYE et al. Aequirea Green Fluorescent Protein Expression of the Gene and Fluorescence Characteristics of the Recombinant Protein. FEBS Letters. 1994, Vol.341, pages 277-280, especially pages 277-279.	11